MOLECULAR DRUG SUSCEPTIBILITY TEST FOR EARLY DIAGNOSIS FOR DRUG RESISTANT TUBERCULOSIS

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ABSTRACT

The rise of drug-resistant tuberculosis poses a severe danger to the global effort to eradicate tuberculosis (DR-TB). Delays in the application of drug susceptibility testing (DST) based on culture led to extended durations of treatment failure and persistent tuberculosis transmission. This article's objective is to give a summary of the several rapid molecular drug susceptibility tests that have proven helpful in promptly identifying DR-TB cases, enabling prompt and efficient treatment of those individuals.

KEYWORDS: Drug resistant tuberculosis (DR-TB), Molecular drug susceptibility test, Xpert MTB/XDR, Whole Genome Sequencing (WGS) and Next Generation Sequencing (NGS)

INTRODUCTION

Despite international efforts, tuberculosis still affects people everywhere and is a major source of disease and mortality. The rise of drug-resistant tuberculosis poses a severe threat to the global effort to eradicate tuberculosis (DR-TB). There are various varieties of DR-TB. Unlike tuberculosis (TB) that is resistant to multiple drugs, which is resistant to more than one first-line anti-TB medication aside from isoniazid(H) and rifampicin (R), isoniazid(H) resistant tuberculosis (TB) refers to tuberculosis cases whose Isoniazid resistance exists in the strain of Mycobacterium tuberculosis (MTB). Tuberculosis that is resistant to R is referred to as "rifampicin-resistant tuberculosis" (RR-TB), either genetically or phenotypically, and may or may not also be resistant to other anti-TB medications. As a stand-in marker for multi-drug resistant tuberculosis (MDR-TB), RR-TB is employed. MDR-TB is caused by MTB strains that are at least resistant to H and R. In addition to being resistant to all fluoroquinolones (FQ) and second-line injectable medications (SLID), extensively drug resistant tuberculosis (XDR-TB) is also known as multidrug resistant tuberculosis (MDR-TB). The definitions of DR-TB have changed throughout time as well. According to an update to the definition made by the WHO in 2021, The term "pre-XDR-TB" refers to tuberculosis (TB) caused by MTB strains that meet the criteria for both MDR/RR-TB and resistance to any form of food poisoning (FQ). On the other hand, "XDR-TB" refers to tuberculosis caused by MTB strains that meet the criteria for both MDR/RR-TB and resistance to any form of food poisoning with at least one additional Group A drug (bedaquiline or linezolid). First (1) The Global Tuberculosis Report 2020 states that 3.3% of newly diagnosed cases and 18% of cases that had previously undergone treatment for tuberculosis were due to rifampicin-resistant and multidrug-resistant tuberculosis (RR/MDR-TB). Estimates indicate that MDR-TB was present in 78% of the 4,65,000 RR-TB cases. Just 2,06,030 cases, or 57.1% of the anticipated 4,65,000 cases of RR/MDR-TB, were discovered in 2019, or 44.3%—were effectively treated after 1,77,099 patients, or 38.1%—were recruited. Globally, XDR-TB, or extensively drug-resistant TB, accounted for 6.2% of all MDR-TB cases. According to estimates, 2.8% and 14% of newly diagnosed and previously treated patients in India had RR/MDR-TB, respectively. A 49% treatment success rate is achieved by treating the projected 1,24,000 new cases of RR/MDR-TB that are reported each year, of which 66,300 (or 53.5%) are discovered and reported, and 56,600 (or 45.5%) start therapy. Although culture-based phenotypic DST is thought to be the most reliable method for diagnosing DR-TB, genotypic molecular methods provide rapid insights into different mutations. While liquid-based culture methods are supposed to be faster, they still take 4-6 weeks to provide results, while DST using

solid media requires 8–12 weeks. The lengthy periods of ineffective and delayed therapy caused by the delays associated with the culture-based diagnostic test (DST) resulted in the continuation of tuberculosis transmission. The purpose of this article is to provide an overview of the different rapid molecular drug susceptibility tests that have been useful in quickly detecting DR-TB.

Rapid molecular drug susceptibility testing (genotypic DST)

Known by another name, nucleic acid amplification tests (NAATs), these tests are based on amplifying a particular genetic region of the MTB complex, typically using Polymerase Chain Reaction (PCR). Molecular tests can detect tuberculosis (TB) and resistance to important anti-TB drugs, including isoniazid (H), rifampicin (R), fluoroquinolones (FQ), and second-line injectable treatments (SLID), faster than the traditional Culture and DST procedures. Furthermore, they are easily available at many levels of the healthcare system. However, these tests cannot be used to determine response to treatment (4).

1. Xpert MTB/RIF

May detect TB and RR-TB simultaneously and quickly using a cartridge-based NAAT (CB-NAAT). It detects DNA sequences unique to the MTB complex as well as mutations in the RNA polymerase beta (rpoB) gene, which is connected to RR. The results are obtained in 90 minutes using raw sputum samples and other bodily fluids, with the least amount of biohazard and minimal technical expertise needed to operate. For optimal performance, the instrument requires a room temperature below thirty degrees Celsius. Four (4)

2. Xpert MTB/RIF ULTRA

Because MTB can be quickly and accurately detected in patient samples, as well as because it can detect Rifampicin resistance in the same test, it has the potential to greatly reduce the number of missed smear-negative TB patients. It may also lessen the overall cost of patient care. Results are available in less than 80 minutes, and Ultra performs better and faster with higher sensitivity in smear-negative TB cases overall. (4) After anti-tuberculosis treatment was initiated, When it came to identifying extra patients with tuberculous meningitis who had either trace or significantly low amounts of germs in their CSF, Xpert Ultra outperformed Xpert MTB/RIF in terms of sensitivity. Thus, it may be said that Xpert Ultra is significantly more sensitive than Xpert for the prompt diagnosis of tuberculous meningitis. (5)

3. Xpert MTB/XDR: With a single test, Xpert MTB/XDR finds mutations associated with resistance to fluoroquinolones (FQ), isoniazid (H), and second-line injectable drugs (SLID), including kanamycin, amikacin, capreomycin, and ethionamide (Eto). The test use high resolution melt technology after a semi-quantitative nested PCR. In less than ninety minutes, the results are available. It is simple to use as an Xpert MTB/RIF and is compatible with current GeneXpert devices that have a 10-color module installed. When combined with additional laboratory and clinical data, it can help diagnose MDR and XDR-TB because it is thought of as a spontaneous test for a sample that is likely to be MTB positive. It will be presented as a test that comes after molecular testing that identify RR and MTB. After the WHO approves it and conducts an in-country assessment and operational feasibility studies, it will be implemented. If FQ resistance is excluded, It might make it easier to get rapid DST, which is necessary before beginning the shorter oral Bedaquiline-containing MDR/RR-TB regimen. (6)

4. THE MOLBIO TRUELAB REAL-TIME QUANTITATIVE MICRO PCR SYSTEM

Truenat MTB and Truenat MTB-Rif Dx are names for micro real-time PCR-based chip-based NAAT for TB and RR detection, respectively. After extracting DNA, the Truenat MTB tests identify MTB in sputum; the results are available in an hour, and Truenat MTB-Rif Dx is employed in a sequential manner for the identification of RR. For primary healthcare settings, it is perfect because it doesn't require air conditioning or a lot of upkeep. (7).

4. LINE PROBE ASSAYS (LPA)

use reverse hybridization and PCR methods to identify medication resistance. The drug resistance profile of an MTB strain is determined by the LPA family of DNA strip-based tests based on the pattern of amplicon binding to probes targeting the most common resistance-associated mutations to first- and second-line anti-TB drugs, as well as to probes targeting the corresponding wild-type (WT) deoxyribonucleic acid (DNA) sequence. The first line (FL-LPA) assesses resistance to isoniazid (H) and rifampicin (R). Rifampicin is ineffective in the event that mutations in the rpoB gene are found, which indicate resistance to the drug. In addition to determining isoniazid resistance, the KatG gene and InhA promoter area can also identify ethionamide (Eto) resistance. Low-level isoniazid resistance is conferred by mutations in the inhA promoter areas, but these mutations also greatly impact sensitivity to ethionamide and prothionamide. In this scenario, isoniazid, ideally at high dosages, may be useful, however in the case of katG mutations, even at high

doses, isoniazid is ineffective. Fluoroquinolone (FQ) and second line injectable medication resistance is determined by second line (SL-LPA) (SLIDs). SLID resistance is caused by mutations in the rrs and eis genes, whereas fluoroquinolone resistance is caused by mutations in the gyrA and gyrB genes. Mutations in the gyrA gene provide resistance to low-level moxifloxacin (Mfx) and levofloxacin (Lfx). If high levels of Mfx and resistance to Lfx are found, it suggests that both Lfx and Mfx are ineffective, even though Mfx can be administered at greater levelsA mutation in the gyrB gene results in resistance to low-level Mfx that is implied or detected; in this scenario, massive dosages of Mfx can be administered while Lfx is ineffective. If rrs gene mutations are found or assumed, this indicates that Amikacin (Am), Kanamycin (Km), and Capreomycin (Cm) are ineffective in this situation. If eis gene mutations are found or inferred, Am and Cm are probably going to be useful, whereas Km isn't going to be useful to take into account. The LPA results are interpreted based on the existence or absence of wild-type (WT) and mutant (MUT) bands. In cases where drug resistance gene areas are known to yield all WT probes but no MUT probes are generated in the corresponding region, the outcome is given as "Resistance not detected." The term "Resistance detected" refers to the development of one or more MUT probes that identify particular mutations imparting drug resistance, independent of the development of WT probes. When one or more WT probes in gene areas known to give drug resistance are not developed and none of the MUT probes in the corresponding region are developed, the term "resistance inferred" is used. The assay's open tube format poses a drawback since it raises the possibility of cross-contamination of the sample and false-positive results. The test must be performed by a qualified specialist who will take the necessary biosafety and contamination control measures. Phenotypic DST is advised in situations when silent mutations could skew clinical interpretation, hence LPA cannot completely replace phenotypic culture techniques. LPA does, however, unquestionably assist in the prompt identification and appropriate treatment of pre-XDR and XDR TB, as well as RR/MDR TB. (8, 4)

5. GENETIC SEQUENCING

Sequencing is the only way to achieve a universal, thorough, and quick DST and stop the spread and evolution of DR-TB.

a. Whole Genome Sequencing (WGS0 is the process of looking through an organism's genetic material's entire DNA sequence. WGS of MTB entails deciphering the precise sequence of every nucleotide that makes up the bacterial genome in order to gather all of its associated data. For M. tuberculosis WGS to provide enough pure mycobacterial DNA, cultured isolates that are either fresh or frozen can be used. Sequencing may be used to help DR-TB patients make treatment decisions, according to recent research conducted in high-burden nations. (9).

b. Next Generation Sequencing (NGS) intends to sequence a particular selection of genes or gene areas that are either known or believed to be linked to a specific feature (like treatment resistance) or disease (like Mycobacterium tuberculosis). Drug-resistant tuberculosis (DR-TB) can be quickly detected using NGS in a variety of clinical reference laboratory scenarios. By providing speedier, The NGS technique solves these challenges by providing complete sequencing evidence for numerous gene regions or entire genomes of interest. Moreover, NGS can be utilized to detect genomic sequence variants that predict drug-resistant tuberculosis phenotypes, identify strain lineages and resistance mechanisms for TB surveillance, and identify genetically linked strains in order to break down transmission networks (9).

While targeted NGS and WGS share the same core NGS workflow and can run on the same NGS instrument, the sample type input requirements and processing steps may differ significantly based on the intended application. TB laboratories at the national and state levels now have five WGS platforms (Illumina Miseq.) and one Pyrosequencer (Quaigen, PyroMark 48) in operation. Initially, these will be employed for drug resistance sentinel surveillance. On both methods, targeted sequencing can be used to identify drug resistance. Targeted sequencing, provided by Illumina, enables the detection of novel mutations in addition to known variants, as detected by pyrosequencing. Over time, algorithms based on sequencing will be developed for clinical management. Although genetic sequencing has the potential to be a quick in vitro diagnosis, its widespread use outside of reference laboratories has been hampered by its expense, complicated workflows, difficult to understand data, and fragile equipment, particularly in low- and middle-income nations. (10).

One of the primary principles of the End TB Strategy is the use of rapid molecular diagnostics to improve tuberculosis therapy and prevention. Thus, rapid molecular DST is required to contain and prevent the transmission of DR-TB. These rapid molecular testing will also allow for minimally delayed, timely, and successful medication for DR-TB.

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